Effects of Vacuum and Controlled Atmosphere Treatments on Insect Mortality and Lettuce Quality

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ABSTRACT   Laboratory studies were conducted to determine the effects of vacuum and controlled atmosphere on mortality of aphids, *Nasonovia ribisnigri* (Mosley) and *Macrosiphum euphorbiae* (Thomas), and leafminer, *Liriomyza langei* Frick, and on the visual quality of iceberg lettuce at three different temperatures. Vacuum at 50 mbar and controlled atmosphere with 6% CO_2 were effective in controlling aphids and leafminer larvae. Complete control of *N. ribisnigri* and *M. euphorbiae* was achieved with vacuum treatments and 6% CO_2 CA treatments at 5°C in 4 d. Mortality was >96% when leafminer larvae were treated with vacuum and 6% CO_2 CA treatments for 4 d. However, leafminer pupae were more tolerant to the treatments and highest mortality was close to 60% in 4 d with CO_2 under vacuum. None of the treatments had negative effects on visual quality of iceberg lettuce. Results from this study are encouraging and warrant further and large-scale research.

KEY WORDS   Controlled atmosphere, vacuum, postharvest pest control, postharvest quality, lettuce

POSTHARVEST INSECT CONTROL is important for export of U.S. lettuce. Live insects are often found in commercial lettuce heads (Hinsch et al. 1991). Importing countries such as Japan with strict phytosanitary regulations will reject or require fumigation at ports of entry with chemicals such as methyl bromide or hydrogen cyanide for lettuce contaminated with live insects. Because these chemicals are injurious to lettuce, the risk of fumigation has deterred most potential export of U.S. lettuce to Japan. With increased restrictions on potential environmental and health effects of pesticides and pesticide resistance problems by pests, it is increasingly difficult to have insect-free lettuce through preharvest pest management (Martin et al. 1995, Rufingier et al. 1997, Barber et al. 1999). Therefore, alternative benign postharvest treatments are needed to solve the insect problem for exported lettuce and to deal with potential pest quarantine problems on lettuce and other fresh products. With increasing trade of agricultural commodities around the globe, such postharvest pest control technology is vital to minimize spread of potentially harmful insects and facilitate global trade and export of U.S. agricultural commodities.

Controlled atmospheres (CA) have been studied extensively for storage of perishable agricultural commodities (Isenberg 1979; Kader 1986; Wang 1990; Raghavan et al. 1996; Kader et al. 1998; Beaudry 1999, 2000; Mitcham et al. 2001). Vacuum storage, also known as hypobaric storage, has been used to extend shelf life of vegetables (Jamieson 1980, Burg and Kos-

son 1983, Gorris et al. 1994, Burg 1990, Knee and Aggarwal 2000). Controlled atmospheres and vacuum storage were also studied for postharvest pest control (Navarro and Calderon 1979; Fleurat-Lessard 1990; Ke and Kader 1992; Whiting et al. 1992; Yi et al. 1992; Carpenter and Potter 1994; Mitcham et al. 1997, 2001). Controlled atmosphere treatments were developed for western flower thrips, pacific spider mites, and omnivorous leafrollers on grapes (Mitcham et al. 1997, Zhou and Mitcham 1998). However, no satisfactory results have been reported for postharvest insect control with CA on lettuce.

There are many studies on effects of CA on lettuce (Lipton 1967, 1971; Lipton et al. 1972; Singh et al. 1972; Brecht et al. 1973a, b, 1973c; Stewart and Uota 1976; Zheng et al. 1993; Cantwell et al. 1995, 1996; Jamie and Saltveit 2002). CA for insect control typically uses high CO_2 concentrations, but lettuce is very sensitive to CO_2. CO_2 as low as 1% could cause injury to susceptible lettuce cultivars (Lipton et al. 1972). Forms of CO_2 injury to lettuce are distinct lesions termed "brown stain" and reddish-orange discoloration of heart leaves (Lipton et al. 1972). There is also considerable variation among different lettuce cultivars in CO_2 sensitivity (Brecht et al. 1973a).

Vacuum is widely used to cool lettuce after harvest and has been studied for postharvest pest control mostly with stored product insects (Bare 1948, Yi et al. 1992, Locatelli and Daolio 1993). Aharoni et al. (1986) demonstrated that a 52 h vacuum treatment of 2.66 kPa (26.6 mbar) at 2°C killed 100% green peach aphid without detrimental effects on lettuce quality. The

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main mechanism for vacuum effects is considered to be lack of oxygen (Navarro and Calderon 1979).

In the current study, vacuum and CA treatments were tested for effects on insect mortality and visual quality of lettuce. Aphids and leafminers are two of insect groups that are frequently intercepted on exported lettuce. Nymphs of two aphid species *Nasonovia ribisnigri* (Mosley) and *Macrosiphum euphorbiae* (Thomas) and larvae and pupae of the leafminer *Liriomyza langei* Frick were tested for responses to vacuum and CA treatments in the current study.

Materials and Methods

Vacuum and CA System. A system that can maintain vacuum and CA conditions was used to conduct vacuum and CA treatments. The system consisted of CO_2 and N_2 gas cylinders, flow meters and gas mixers, solenoid valves, programmable timer, vacuum pump, three refrigerators, and treatment chambers. Treatment chambers were made of modified eight quart pressure cookers (National Presto Industries, Eau Claire, WI) and housed in three refrigerators (Lab-Line 3766, Lab-Line Instruments, Melrose Park, IL) set at 0, 5, or 10°C with variation of ±1°C. A glass thermometer was placed in each refrigerator for monitoring temperature. Each treatment chamber had an inlet and outlet gas line. CO_2 and N_2 at 20 psi first passed through solenoid valves to reach rotameters and/or gas proportioning rotameters (Omega Engineering, Stamford, CT) for mixing. Each of the outlets from rotameters and gas proportioning rotameters were connected using vinyl tubing (0.635 cm) to a reservoir chamber, similar to the treatments chambers, located on top of one of the refrigerators. The reservoir chamber was linked through vinyl tubing to three or six treatment chambers in the three refrigerators depending on the experimental design. The gas outlets from treatment chambers belonging to one treatment from all three refrigerators were linked with vinyl tubing to a solenoid valve and then to a vacuum pump (model E2M1.5, BOC Edwards, Wilmington, MA). A CO_2 analyzer was used to measure CO_2 concentrations (model 302M, NOVA Analytical Systems, Niagara Falls, NY). A programmable timer (Chron-Trol XT, ChronTrol Corp., San Diego, CA) was used to control the solenoid valves. A vacuum switch (PSW 541, Omega Engineering, Stamford, CT) was connected to each reservoir chamber and linked to the solenoid valve on the outlet line for maintaining preset vacuum levels in treatment chambers. Quick couplings with shutoff valves were used to allow each individual treatment chamber to be disconnected without affecting the atmospheres of the other chambers. Vacuum levels in the reservoir chambers were measured with a vacuum test gauge (Ashcroft type 1082, Dresser Instrument, Stratford, CT). After placing test subjects in the treatment chambers, the air was removed from all chambers by running the vacuum for 20 min, resulting in a pressure of <50 mbar. The purpose was to minimize residual O_2 in treatment chambers and their effects on experiments.

Treatments. Two to four treatments and one control were tested at 0, 5, and 10°C in three refrigerators depending on the experimental design. The four treatments used were: (1) absolute pressure of ≈50 mbar with injection of N_2 gas of 1–1.5 liters every 30 min; (2) absolute pressure of ≈50 mbar with injection of CO_2 gas of 1–1.5 liters every 30 min; (3) controlled atmosphere of 100% N_2 gas at atmospheric pressure; and (4) controlled atmosphere of 6% CO_2 in N_2 at atmospheric pressure. Test subjects were placed on refrigerator shelves to serve as controls. In treatments 1 and 2, absolute pressure (referred as vacuum hereafter) of 50 mbar means that treatment chambers maintained equivalent of gas of 5% volume of treatment chambers at normal pressure. The injection of 1–1.5 liters of N_2 or CO_2 every 30 min was equivalent to replacing all air in treatment chambers once in less than an hour. In treatments 3 and 4, treatment chambers were filled with CA back to atmospheric pressure after 20 min vacuum (<50 mbar) to remove air from all chambers. This process was repeated every 3 h to renew the CA in treatment chambers. CO_2 concentration was measured every day with the CO_2 analyzer for treatments three and four in treatment chambers in the three refrigerators. CO_2 concentration was 0 for treatment three and ranged between 5.4 and 6.0% for treatment four in all experiments.

Insects. Two aphid species, the currant-lettuce aphid, *Nasonovia ribisnigri* (Homoptera: Aphididae) and potato aphid, *Macrosiphum euphorbiae* (Homoptera: Aphididae), and the leafminer, *Liriomyza langei* Frick (Diptera: Agromyzidae) were used in this study. *N. ribisnigri* colonies were established from *N. ribisnigri* collected in 2001 in Spence fields, Salinas, CA. *M. euphorbiae* colonies were established from naturally infested lettuce plants in the greenhouse. Both *N. ribisnigri* and *M. euphorbiae* colonies were maintained on romaine and iceberg lettuce (cultivar Salinas) in screen cages in the greenhouse. Leafminer-infested lettuce leaves collected from newly harvested lettuce fields were used as the sources of leafminer larvae and pupae.

Experiments with Aphids. For experiments with aphids, 10 medium-sized nymphs of *N. ribisnigri* or *M. euphorbiae* were transferred with a soft brush from infested colony plants onto a piece of romaine lettuce leaf in a plastic petri dish. For each treatment x temperature combination (one treatment chamber), 2–6 petri dishes were stacked and placed in a 17.8 × 20.3 cm zip-lock bag. The petri dishes have tops and bottoms separated by small spaces to allow gas exchange. The zip-lock bag was punched twice below the zip line to form four 7-mm holes for ventilation. A plastic petri dish filled with water-soaked paper towel was placed on top of the petri dish stack to maintain high humidity. The zip-lock bag was sealed and then fastened with a rubber band to prevent accidental openings of petri dishes in the bag that could result in crawling out of aphids from the petri dishes. The bag was then placed in a treatment chamber to be treated. Most leaves in the petri dishes remained turgid at the end of treatment except those in vacuum treatments at 10°C. After

treatment, zip-lock bags with petri dish stacks were transferred to an environmental chamber held at 24°C and 14:10 (L:D) photoperiod without rubber bands. Mortality of aphids was scored after 1 d under an illuminated magnifying glass. Aphids that failed to stand or move appendages in response to repeated probes were classified as dead.

In the first set of experiments, four treatments were tested against *N. ribisnigri* nymphs at 0, 5, and 10°C for 2, 3, and 4 d. Controls were also included for each treatment time at each temperature. The experiment was replicated twice. In each experiment, six petri dishes, each with 10 *N. ribisnigri* nymphs, were enclosed in a zip-lock bag with ventilation holes for each treatment × temperature combination. After 2, 3, and 4 d, the experiment was paused so that treatment chambers could be disconnected individually and returned to atmospheric pressure. Two petri dishes were removed from each chamber at each of the three dates. The experiment resumed with the remaining petri dishes. The petri dishes removed from treatment chambers were enclosed in a zip-lock bag and held in the environmental chamber as stated above and mortality of the insects in those petri dishes was scored after 1 d. Then fresh leaves were added to petri dishes and mortality was recorded again after another 2 d (3 d after treatment). Because CO_2 has an anesthetic effect on insects, surviving insects after CA treatments need time to recover. The purpose of scoring mortality of *N. ribisnigri* twice was to make sure that mortality would not decline from 1 to 3 d after treatment, indicating recovery of insects. Because no additional recovery occurred after 1 d, in subsequent experiments with aphids, mortality was recorded only at 1 d after the end of treatment.

Two treatments, vacuum + CO_2 and CA of 6% CO_2, were further tested against *N. ribisnigri* nymphs in two 4 d continuous tests at 0, 5, and 10°C without stopping in the middle as in the first set of experiments. Controls were also included. A total of ≈40 *N. ribisnigri* nymphs in four petri dishes were tested for each treatment at each temperature. Mortality was recorded 1 d after the end of treatment. Three treatments, vacuum + N_2, vacuum + CO_2, and CA of 6% CO_2, were tested against *M. euphorbiae* nymphs in two 4 d tests at 0, 5, and 10°C. Controls were also included. A total of 27–78 *M. euphorbiae* nymphs in 2–4 petri dishes were tested for each treatment at each temperature. Mortality was recorded 1 d after the end of treatment.

Experiments with Leafminer. For experiments with leafminer larvae, leafminer infested lettuce leaves were collected from newly harvested lettuce fields and were randomly divided into groups of five leaves and placed in plastic produce bags (36 cm × 28 cm) and weighed on an electronic balance. Ten bags were used as checks and were suspended in ten plastic buckets (37 cm high × 30 cm diameter) with metal wires piercing through bags and leaves and resting on the rims of the buckets. Pupae were collected at day 7 and 14 from each bucket to determine total pupae production from each group of five leaves. Regression between leaf weight and number of pupae was estab-

lished using leaf weights and pupal productions in the checks. The regression line was used to estimate the number of pupae expected from each leaf bag used in treatments assuming no treatment effects. For each treatment x temperature combination, four bags of leaves were placed in two treatment chambers in each refrigerator to be treated with openings of bags folded and taped at the centers to allow gas exchange while minimizing moisture loss. The same numbers of leaf bags were also placed on shelves of refrigerators as controls. After treatment, leaf bags were suspended in plastic buckets as stated above to collect leafminer pupae. The number of pupae collected from each bag after treatment were compared with the number of expected pupae estimated from the regression line to estimate mortality of leafminer larvae caused by treatments.

For experiments with leafminer pupae, field collected leafminer-infested lettuce leaves were suspended in plastic buckets and pupae were collected twice a week. Pupae 2–7 d old were randomly placed in petri dishes (30 pupae/petri dish) fastened with masking tape. For each treatment x temperature combination, 2–4 petri dishes each with 30 leafminer pupae were placed in a treatment chamber to be treated and a similar number of petri dishes were placed on shelves of refrigerators as controls. After treatment, all petri dishes with leafminer pupae were placed in the environmental chamber at 24°C and 14:10 (L:D) photoperiod for three weeks or longer until all emerged adults died. The number of dead leafminer adults in each petri dish was then counted to estimate mortality of pupae.

Two 4 d tests were conducted to determine effects of vacuum and CA on mortality of leafminer larvae and pupae at 0, 5, and 10°C. Three treatments, vacuum + N_2, vacuum + CO_2, and CA of 6% CO_2, were used in each test. A total of 4–8 bags of lettuce leaves were used for each treatment at each temperature in the two tests. A total of 180–360 pupae were used for each treatment at each temperature in the two tests.

Lettuce Quality. Two cultivars of iceberg lettuce, Salinas and Hallmark, were used for evaluating effects of vacuum and CA treatments on lettuce quality. Cultivar Salinas was grown in a field plot at USDA Research Station in Salinas, CA. Lettuce heads were harvested in the morning on the day of experiments and wrapped in commercial perforated plastic bags, labeled and placed in treatment chambers. Lettuce heads of cultivar Hallmark were obtained directly from harvest machines in commercial fields of Tanimura & Antle Co. (Salinas, CA) on the day of experiments. Heads were taken randomly, labeled, and placed in treatment chambers after elimination of heads with severe mechanical injuries. For controls, lettuce heads were placed on shelves of the same refrigerators holding treatment chambers. After treatment, lettuce heads were packed in commercial lettuce boxes and placed in a walk-in cold room at 5°C. After 14 d of posttreatment storage, lettuce heads were removed from the cold room and visual quality of

Table 1. Mortality of *N. ribisnigri* nymphs in response to 2, 3, and 4 d vacuum and controlled atmosphere treatments at three temperatures

Temp (°C)	Treatment	Treatment time (day)					
		2		3		4	
		1 day	3 day	1 day	3 day	1 day	3 day
0	Control	5.0 ± 5.0b	19.1 ± 0.9b	9.1 ± 9.1a	9.1 ± 9.1a	0c	10.0 ± 10.0b
	CA (100% N_2)	11.4 ± 6.8b	11.4 ± 6.8c	10.0 ± 7.1a	10.0 ± 7.1a	10.0 ± 4.1bc	20.3 ± 3.9ab
	CA (6% CO_2)	24.8 ± 6.6a	35.0 ± 2.9b	27.3 ± 8.7a	68.6 ± 7.8a	51.4 ± 14.5ab	70.1 ± 10.6a
	Vacuum + N_2	9.6 ± 6.4b	35.7 ± 20.6b	27.5 ± 17.0a	48.3 ± 19.1a	37.5 ± 16.5ab	62.5 ± 18.9a
	Vacuum + CO_2	31.8 ± 5.0a	55.0 ± 6.5a	35.0 ± 14.4a	70.0 ± 12.2a	60.3 ± 11.5a	77.2 ± 12.4a
5	Control	0b	5.0 ± 5.0b	5.0 ± 5.0b	15.0 ± 5.0b	10.0 ± 10.0c	15.0 ± 5.0b
	CA (100% N_2)	2.3 ± 2.3b	5.8 ± 3.5b	10.0 ± 4.1b	15.3 ± 2.7b	10.0 ± 4.1c	25.0 ± 11.9b
	CA (6% CO_2)	47.5 ± 10.3a	77.5 ± 11.1a	49.4 ± 12.4a	82.4 ± 11.1a	61.7 ± 10.6b	87.1 ± 7.1a
	Vacuum + N_2	49.3 ± 11.3a	71.7 ± 12.6a	62.5 ± 2.5a	85.0 ± 6.5a	67.5 ± 10.3b	90.0 ± 4.1a
	Vacuum + CO_2	28.0 ± 8.0a	85.2 ± 6.5a	55.0 ± 5.0a	92.5 ± 2.5a	93.3 ± 6.7a	100a
10	Control	0c	25.0 ± 5.0c	0d	20.0 ± 0.0b	10.0 ± 0.0b	20.0 ± 10.0b
	CA (100% N_2)	23.6 ± 8.4b	41.1 ± 8.2bc	18.6 ± 9.5c	28.4 ± 8.1b	12.3 ± 6.3b	31.8 ± 2.8b
	CA (6% CO_2)	42.5 ± 11.1b	67.5 ± 16.5abc	70.0 ± 17.8b	90.0 ± 10.0a	90.5 ± 5.5a	97.5 ± 2.5a
	Vacuum + N_2	95.0 ± 2.9a	100a	100a	100a	100a	100a
	Vacuum + CO_2	85.1 ± 6.1a	92.7 ± 4.5ab	100a	100a	100a	100a

For each treatment combination, about 40 *N. ribisnigri* were tested in 4 Petri dishes. A total of 1440 *N. ribisnigri* were tested and 180 *N. ribisnigri* were used in controls. Percent mortality was determined 1 and 3 d after treatment for each Petri dish. Mortality data were transformed by arcsine$\sqrt{x}$ prior to ANOVA. For each temperature, values within each column followed by the same letter were not significantly different, Ryan-Einot-Gabriel-Welsch multiple range test, P > 0.05 (SAS GLM procedure, SAS Institute 1999).

lettuce heads was scored using the scoring system by Kader et al. (1973).

Two tests were conducted for each lettuce cultivar. In each test, three treatments: vacuum + N_2, vacuum + CO_2, and CA of 6% CO_2, were used to treat lettuce continuously for 4 d at 0, 5, and 10°C. One or two lettuce heads were used for each treatment × temperature combination in each test. A total of 7–8 lettuce heads from the two cultivars were tested for each treatment at each temperature. A total of 11 lettuce heads were used as controls at each temperature.

Data Analysis. Mortality for aphids and leafminer pupae were calculated for each petri dish. For leafminer larvae, regression analyses (SAS Institute 1999) were used to establish regression between leaf weights and number of pupae to estimate expected pupae in leaves used in treatments. Mortality rate was estimated for each bag based expected numbers of pupae and numbers of pupae collected after treatment. All mortality data were transformed by arcsine$\sqrt{x}$ and analyzed using analysis of variance (ANOVA) (SAS GLM procedures, SAS Institute 1999). ANOVA was also used to analyze visual quality scores of lettuce heads using SAS GLM procedure (SAS Institute 1999). Ryan-Einot-Gabriel-Welsch multiple range test was used to compare means (SAS Institute 1999).

Results

Aphids. Controlled atmosphere of 6% CO_2 and the two vacuum treatments caused significant higher mortality of *N. ribisnigri* for all three treatment times at 5 and 10°C as compared with controls (Table 1). Complete control of *N. ribisnigri* was achieved with the two vacuum treatments at 10°C for 3 and 4 d. CA of 100% N_2 at normal pressure did not cause significant mortality of *N. ribisnigri* under most combinations of temperatures and time except at the 1 d observation for

the 2 and 3 d treatments at 10°C. For all treatments, mortality scored 3 d after treatment was higher than mortality scored 1 d after treatment. All three effective treatments caused similar levels of mortality of *N. ribisnigri* and there were no significant differences among them under most treatment conditions (Table 1). In the continuous 4 d tests against *N. ribisnigri* nymphs, mortality reached 85% for vacuum + CO_2 and CA with 6% CO_2 at 0°C. Total control of *N. ribisnigri* was achieved with both the treatments at 5 and 10°C (Table 2). For *M. euphorbiae,* 4 d exposure to the three effective treatments, CA of 6% CO_2, vacuum + CO_2, and vacuum + N_2 caused 100% mortality of *M. euphorbiae* at 5 and 10°C and 86–100% mortality at 0°C (Table 2).

Table 2. Mortality of *N. ribisnigri* and *M. euphorbiae* nymphs in response to 4 d continuous treatments of vacuum and controlled atmospheres at three temperatures

Temp (°C)	Treatment	*N. ribisnigri*		*M. euphorbiae*	
		N	Mortality ± SE (%)	N	Mortality ± SE (%)
0	Control	40	10.0 ± 5.8b	59	1.3 ± 1.3b
	CA (6% CO_2)	41	85.1 ± 6.1a	63	95.8 ± 4.2a
	Vacuum + CO_2	39	86.1 ± 8.3a	41	86.2 ± 9.7a
	Vacuum + N_2			36	100a
5	Control	38	13.1 ± 2.3b	54	1.8 ± 1.8b
	CA (6% CO_2)	41	100a	67	100a
	Vacuum + CO_2	39	100a	35	100a
	Vacuum + N_2			43	100a
10	Control	44	6.4 ± 3.8b	76	0b
	CA (6% CO_2)	41	100a	78	100a
	Vacuum + CO_2	41	100a	27	100a
	Vacuum + N_2			32	100a

Percent mortality was determined 1 d after treatment for each Petri dish. Mortality data were transformed by arcsine$\sqrt{x}$ prior to ANOVA. For each temperature, values within each column followed by the same letter were not significantly different, Ryan-Einot-Gabriel-Welsch multiple range test, P > 0.05 (SAS GLM procedure, SAS Institute 1999).

Table 3. Effects of 4 d continuous vacuum and controlled atmosphere treatments on mortality of leafminer larvae

Temp (°C)	Treatment	Total number of pupae		Pupae per gram of leaf		Mortality (%)[a]
		Expected	Collected	Expected	Collected	
0	Control	423	426	1.32 ± 0.02ab	1.36 ± 0.13a	6.0 ± 4.0c
	CA (6% CO_2)	431	100	1.29 ± 0.01b	0.34 ± 0.12c	73.5 ± 8.9a
	Vacuum + N_2	260	146	1.30 ± 0.003b	0.73 ± 0.15bc	44.5 ± 11.5b
	Vacuum + CO_2	202	132	1.38 ± 0.003a	0.90 ± 0.02b	34.5 ± 1.5b
5	Control	402	357	1.32 ± 0.003a	1.17 ± 0.09a	11.7 ± 6.0b
	CA (6% CO_2)	442	20	1.26 ± 0.03a	0.06 ± 0.01c	95.0 ± 0.8a
	Vacuum + N_2	246	7	1.31 ± 0.002a	0.04 ± 0.03c	97.0 ± 2.0a
	Vacuum + CO_2	166	86	1.30 ± 0.03a	0.66 ± 0.26b	50.0 ± 18.0b
10	Control	368	261	1.27 ± 0.03a	0.90 ± 0.17a	30.3 ± 12.8b
	CA (6% CO_2)	342	3	1.25 ± 0.08a	0.01 ± 0.01b	99.3 ± 0.7a
	Vacuum + N_2	228	1	1.31 ± 0.003a	0.01 ± 0.01b	99.5 ± 0.5a
	Vacuum + CO_2	192	7	1.35 ± 0.03a	0.05 ± 0.02b	96.5 ± 1.5a

For each temperature, values within each column followed by the same letter were not significantly different, Ryan-Einot-Gabriel-Welsch multiple range test, P > 0.05 (SAS GLM procedure, SAS Institute 1999).

[a] Percent mortality rate of leafminer larvae was estimated for each bag based on expected numbers of pupae assuming no treatment effects and numbers of pupae collected after treatment. Mortality data were transformed by arcsine$\sqrt{x}$ prior to ANOVA.

Leafminer. All three treatments of 4 d had significant effects on survival of leafminer larvae in lettuce leaves (Table 3). This was indicated by significant differences between controls and treatments in larval mortality and number of pupae collected per gram of leaf. At 10°C, all three treatments caused close to 100% mortality of leafminer larvae and there were no significant differences among the three treatments. At 0°C, there were differences among the three treatments in larval mortality and CA with 6% CO_2 caused higher mortality than the two vacuum treatments. At 5°C, however, vacuum + CO_2 resulted in lower mortality than CA with 6% CO_2 or vacuum + N_2 (Table 3). The total number of pupae expected, assuming no treatment effects, ranged from 166 to 442. The actual collected pupae were very low for the three treatments at 10°C ranging from 1 to 7. The expected pupae per gram of leaf tissue remained similar although significant differences were found among treatments including the control at 0°C. All three treatments yielded significantly lower number of pupae per gram of leaf compared with the control at all three temperatures. However, there were also significant difference among the three treatments in pupae collected per gram of leaf or larval mortality for 0 and 5°C. Number of leafminer pupae collected from leaves correlated significantly with leaf weight for one experiment ($y = 1.836x − 33.757$, $R^2_{adj} = 0.748$, $P < 0.001$) but not for the other ($y = 1.244x + 5.930$, $R^2_{adj} = 0.061$, $P = 0.219$).

The three treatments also caused moderate mortality of leafminer pupae but effects were variable (Table 4). Significant differences between the control and treatments in pupal mortality were detected at 0 and 10°C, but no significant difference was found at 5°C. There were also high mortality rates of pupae in controls. After correcting for control mortality using Abbott's formula, the mortality of pupae for the three treatments reached 37–59% at 10°C.

Lettuce Quality. None of the treatments significantly reduced visual quality of lettuce heads (Table 5). The scores of visual quality indicated marketability of lettuce heads. The higher the score, the better the quality. A score of 7, 5, and 3 indicated visual quality to be good, fair, and poor, respectively (Kader et al. 1973). Most treatment × temperature combinations had visual quality scores of ≥5. There were no significant differences in visual quality among treatments and controls at 5 and 10°C. At 0°C, visual quality of untreated lettuce heads was lower than that for treated heads and the difference was significant between heads in the control and vacuum + CO_2 treatment (Table 5).

Discussion

Significant effects of vacuum and CA treatments on mortality of *N. ribisnigri*, *M. euphorbiae*, and leafminer larvae indicate that CA treatments with low CO_2 concentrations and extremely low O_2 or no O_2 and vacuum treatments had potential to kill insects without

Table 4. Effects of 4 d continuous vacuum and controlled atmosphere treatments on leafminer pupal mortality at three temperatures

Temp (°C)	Treatment	N	Mortality (%)	Corrected mortality (%)
0	Control	180	48.3 ± 11.7b	
	CA (6% CO_2)	360	66.1 ± 2.6a	34.4
	Vacuum + N_2	180	64.4 ± 3.4a	31.1
	Vacuum + CO_2	180	61.1 ± 3.7a	24.8
5	Control	180	53.3 ± 3.4a	
	CA (6% CO_2)	360	60.0 ± 2.8a	14.3
	Vacuum + N_2	180	63.9 ± 3.2a	22.7
	Vacuum + CO_2	180	62.2 ± 5.1a	19.1
10	Control	180	52.8 ± 4.5c	
	CA (6% CO_2)	360	73.6 ± 2.3b	44.1
	Vacuum + N_2	180	70.6 ± 3.3b	37.7
	Vacuum + CO_2	180	80.6 ± 2.8a	58.9

Percent mortality was determined for each Petri dish. Mortality data were transformed by arcsine$\sqrt{x}$ prior to ANOVA. For each temperature, values within each column followed by the same letter were not significantly different, Ryan-Einot-Gabriel-Welsch multiple range test, P > 0.05 (SAS GLM procedure, SAS Institute 1999). Corrected mortality was calculated using Abbott's formula.

Table 5. Effects of 4 d continuous vacuum and controlled atmosphere treatments on lettuce quality at three temperatures after 2 wk post-treatment storage at 5°C

T (°C)	Treatment	N	Visual quality (mean ± SE)
0	Control	11	4.3 ± 0.6b
	CA (6% CO_2)	8	5.1 ± 0.8b
	Vacuum + N_2	8	5.9 ± 0.6ab
	Vacuum + CO_2	8	6.9 ± 0.1a
5	Control	11	5.2 ± 0.5a
	CA (6% CO_2)	8	5.8 ± 0.5a
	Vacuum + N_2	8	6.4 ± 0.6a
	Vacuum + CO_2	8	6.1 ± 0.6a
10	Control	11	5.1 ± 0.5a
	CA (6% CO_2)	7	5.7 ± 0.6a
	Vacuum + N_2	7	3.6 ± 0.8a
	Vacuum + CO_2	8	5.3 ± 0.7a

For each temperature, values within each column followed by the same letter were not significantly different, Ryan-Einot-Gabriel-Welsch multiple range test, $P > 0.05$ (SAS GLM procedure, SAS Institute 1999). Visual quality ranges from 1 (extremely poor) to 9 (excellent), with 5 as fair, with slightly to moderately objectionable defects (Kader *et al.* 1973).

negative effects on visual quality of lettuce. The three insects used in the current study have not been subjected to CA or vacuum treatments in previous research. Therefore, comparisons with previous research should be viewed with caution. A previous study with higher CO_2 (10%) CA failed to achieve complete control of unspecified aphids with much longer treatments (14 d) at 5°C (Zheng et al. 1993). Incomplete control of green peach aphid was achieved with 30% CO_2 and 5% O_2 in 40 h at 12°C (Epenhuijsen et al. 2002). In comparison, complete control of *N. ribisnigri* and *M. euphorbiae* was achieved with 6% CO_2 in 4 d at 5°C in the current study. The crucial differences may include the use of vacuum initially to remove air in the system in our study. Periodic vacuum followed by filling treatment chamber with 6% CO_2 in nitrogen helps to remove residual O_2 in the system. In an earlier study with New Zealand flower thrips, mortality was significantly higher when there was no O_2 than when there was 0.25% O_2 (Carpenter et al. 1996). That research and results of the current study point out the importance of maintaining extremely low or no O_2 CA conditions for pest control. The effect of temperature on insect response to CA found in this study was also reported for other pests such as thrips (Potter et al. 1994) and European red mite (Lidster et al. 1984). Whiting et al. (1992) also concluded that reducing O_2 and increasing temperature enhanced CA efficacy more than did increasing CO_2 levels for control of light brown apple moth and codling moth. Our results, together with these earlier studies, show good potential of CA with low CO_2 level and extreme low or no O_2 for postharvest pest control on lettuce. The relatively high tolerance of lettuce to low O_2 is also an important factor favoring this approach. The effectiveness of low CO_2 CA derives from synergistic interactions between CO_2 and O_2 levels in affecting insect survival. At an extremely low O_2 levels, slight increases in CO_2 were shown to greatly reduce

the treatment time needed to achieve the same level of control of red flour beetle, *Tribolium castaneum*, adults (Calderon and Navarro 1979, Fleurat-Lessard 1990). However, extremely low O_2 levels may also aggravate CO_2 injuries to lettuce (Brecht et al. 1973c).

The lack of any negative impact on lettuce quality by CA and vacuum treatments suggest that the treatments may be tolerated by lettuce even at 10°C. The visual quality score of lettuce measures general appearance of lettuce associated with marketability to consumers. It incorporates many factors including defects such as brown stain caused by CO_2. But brown stain was not a major factor in this study. Based on similar visual quality scores for the control and treatments at 5 and 10°C, it is possible to develop effective CA treatments at 10°C without significant degradation to lettuce quality. A previous study by Brecht et al. (1973b) showed that the brown stain index of head lettuce actually declined from 56 to 1 after CA treatments of 2% O_2 and 5% CO_2 when temperature increased from 0 to 10°C. This lends further support for developing CA treatments at warmer temperatures such as 10°C.

The primary mechanism of action of vacuum treatment on insect survival is considered to be lack of O_2 (Navarro and Calderon 1979). However, CA with 100% N_2 was not effective against *N. ribisnigri* while vacuum + N_2 was effective. This difference could be a result of lower O_2 in vacuum treatment than in 100% N_2 CA treatment if the N_2 has residual O_2 although we could not detect O_2 in N_2 supplies. It is also possible that vacuum treatment may contribute to insect mortality by other mechanisms. In addition to lack of O_2, vacuum is also suggested to cause insect mortality through dehydration and direct effects (Galun and Fraenkel 1961). The effectiveness against leafminer larvae may also help to clarify the mechanism of vacuum effects. Because leafminer larvae feed internally in leaves, a moist ambient condition was likely maintained during treatment. Therefore, dehydration, suggested as a major contributing factor for insect mortality in response to vacuum treatments, is unlikely to be a cause for the high mortality rates of leafminer larvae. For aphids, large surface to volume ratio because of their small body sizes is likely to make them prone to dehydration. Leaves in vacuum treatments at 10°C also wilted by the end of treatment. Therefore, dehydration effects cannot be ruled out even moist paper towel was provided to maintain moist conditions in petri dishes.

N. ribisnigri mortality scored 3 d after treatment was equal or higher than the mortality scored 1 d after treatment. This suggests that there was little if any recovery of aphids between 1 and 3 d after treatment. Therefore mortality scored 1 d after treatment was a conservative measure of treatment effectiveness. Because of complications of reproduction by surviving aphids after the treatments, mortality scored 1 d after treatment was used for other experiments with aphids.

Comparing responses of *N. ribisnigri* in the first experiment in which the treatment was interrupted each day to get mortality data at 2 and 3 d and the

second experiment in which treatments were continuous, mortality for continuous treatment of 6% CO_2 CA reached 100% compared with 87% for noncontinuous treatment of 6% CO_2 CA (Tables 1 and 2). This difference is likely due to exposure of insects to normal air in the noncontinuous treatments, given the importance of maintaining extreme low O_2 to kill insects. The lower mortality for vacuum + CO_2 than those for CA with 6% CO_2 or vacuum + N_2 at 5°C (Table 3) was likely a result of a leak in the treatment chamber. The leak was confirmed after the experiment by decline of vacuum level overnight when the chamber was connected to the vacuum test gauge and isolated under pressure of <50 mbar.

In leafminer experiments, lettuce leaves infested by leafminers came directly from the field and likely contained eggs and larvae at various stages of development. Because larvae exit from infested leaves as they mature and some larvae pupate on leaves, it is possible that a few pupae collected after the treatments might be due to pupation of mature larvae that exited leaves before treatment effects began or to pupae stuck to the leaf surfaces. Therefore, mortality rates obtained in the experiments were likely conservative. The effectiveness of low CO_2 CA and vacuum treatments on leafminer larvae indicate that insects that feed internally were also susceptible to CA and vacuum treatments.

Results of controlling aphids and leafminer larvae while preserving lettuce quality by both vacuum and CA treatments are encouraging and warrant further research. However, practical use of high vacuum for postharvest insect control on lettuce or other vegetables is more difficult than controlled atmosphere because of the need of vacuum chambers. Therefore, future efforts should be focused on large scale studies with controlled atmosphere with low CO_2 concentrations to explore the possibility of practical applications of the controlled atmosphere treatments for postharvest pest control on lettuce.

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